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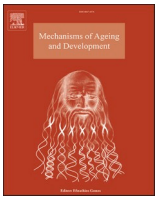

Current senolytics: Mode of action, efficacy and limitations, and their future

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ABSTRACT

Senescence is a cellular state characterized by its near-permanent halted cell cycle and distinct secretory phenotype. Although senescent cells have a variety of beneficial physiological functions, progressive accumulation of these cells due to aging or other conditions has been widely shown to provoke deleterious effects on the normal functioning of the same or higher-level biological organizations. Recently, erasing senescent cells in vivo, using senolytics, could ameliorate diseases identified with an elevated number of senescent cells. Since then, researchers have struggled to develop new senolytics each with different selectivity and potency. In this review, we have gathered and classified the proposed senolytics and discussed their mechanisms of action. Moreover, we highlight the heterogeneity of senolytics regarding their effect sizes, and cell type specificity as well as comment on the exploited strategies to improve these features. Finally, we suggest some prospective routes for the novel methods for ablation of senescent cells.

1. Introduction

Senescence is a cellular state in which the cells resist undergoing replication even in exposure to replication forces (He and Sharpless, 2017). The epigenetic remodeling due to senescence induction gives the senescent cells unique characteristics that are not temporary and generally remain with them till their death (Zhu et al., 2021). Their cell cycle arrest usually comes with an increase in p16 and/or p21 expression, which eventually through the inhibition of the release of the retinoblastoma protein (RB) family from E2F, does not allow the cell cycle exit, which is executed by activating E2F targets (Kumari and Jat, 2021). Senescent cells possess a specific secretary phenotype, named senescence-associated secretory phenotype (SASP), which remodels their surrounding niche. This includes multiple inflammatory cytokines/factors (e.g. IL1A, IL1B, IL6, GM-CSF), chemokines (e.g. CXCL1, CXCL2), growth factors (e.g. AREG, EGF, FGF1, VEGFA), and proteases (e.g. MMP1, MMP3, MMP10, MMP12)(Coppé et al., 2010). This phenotype has benefits in wound healing, embryogenesis, and cancerogenesis surveillance (Huang et al., 2022b); However, the accumulation of senescent cells in a tissue above a threshold vastly alters its normal function (Xu et al., 2018). Senescent cell burden usually elevates with age, furthermore, there are some other factors that contribute to senescent cell accumulation including, impaired immune clearance of these cells, increased cellular stressors spanning reactive oxygen species over-production, DNA damage agents (including chemotherapeutics), and proteotoxic stresses (Childs et al., 2015). Senotherapeutics are a new generation of drugs that are seeking to target the deleterious effect of senescent cells. Generally, two different strategies for senotherapy have been proposed; one is to inhibit the SASP; these drugs are called senomorphics. The other strategy is to selectively remove these cells without killing non-senescent cells, a process now termed senolysis and the corresponding drugs senolytics (Zhang et al., 2022a). A third approach which is still in its infancy has been discussed recently; It was named senoreversal, an approach that seeks reprogramming of senescent cells to their normal original state (Zhang et al., 2022b). Accumulation of senescent cells has been demonstrated in a variety of diseases including cardiovascular diseases e.g. atherosclerosis (Childs et al., 2016), neurodegenerative diseases including Alzheimer's (Zhang et al., 2019), osteoarthritis (Coryell et al., 2021), osteoporosis (Farr et al., 2017), idiopathic pulmonary fibrosis (Schafer et al., 2017), hepatic

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steatosis (Ogrodnik et al., 2017), and Trisomy 21 (Meharena et al., 2022) in which their selective ablation exhibited promising results in animal studies. The term senolytic was first coined by the discovery of dasatinib and quercetin that by inhibiting pro-survival pathways in senescent cells, selectively killed these cells; however, the lethality of each of these compounds was cell-specific, therefore the authors by combining these two drugs, provoked a broader cell-type range of senolytic efficacy (Zhu et al., 2015). Since then, there have been many efforts to discover new senolytics through large unbiased screening or rational, hypothesis-driven analyses of compounds or proteins. Some of these senolytics have now reached clinical trials (Chaib et al., 2022). In this review, we are going to delve into the molecular mechanisms of the senolytics identified up to now and compare their efficacy variations depending on cell type and senescence trigger alongside evaluating the proposed solutions to enhance their efficacy. Moreover, we are going to suggest some prospective pathways and targets which might have roles in the protection or vulnerabilities of senescent cells for future discovery of the next generations of senolytics.

2. Molecular mechanisms of senolysis

By overviewing the available studies on the discovery of senolytics, we can generally classify these efforts into two different groups (although there might be some overlaps and no boundary can fully segregate them). Many researchers have focused on the protective targets and the pro-survival pathways which have been established by the induction of senescence in cells; suppressing these targets should be lethal in senescent cells but preferably should not be harmful to non-senescent cells. Presumably targeting the pro-survival pathways most likely contribute to apoptosis but not the other forms of cell death like necrosis and ferroptosis since they have a marginal effect on other cellular pathways including the production of ATP which is essential for apoptosis. While senescent cells lose their efficiency in controlling the perfect cellular homeostasis and lose many of their repairing capacities, other researchers have concentrated on these vulnerabilities; Small stresses in fragile points can contribute to the destabilization and the death of senescent cells while these stresses might be coped easily by non-senescent cells. In contrast to the inhibitors of protective factors, such stressors (besides induction of apoptosis), might also imply other forms of cell death e.g., necrosis or ferroptosis. These forms of cell death might be accompanied by more off-target side effects due to inducing a general inflammatory response.

2.1. Inhibiting protective factors

2.1.1. Manipulation of the upstream regulators of apoptosis

The idea of the first study of senolytics was shaped with the goal to disrupt the pro-survival pathways in senescent cells. Two of the imperative targets for the survival of senescent cells were determined as ephrins (EFNB-1/3) and phosphatidylinositol 3-kinases (PI3K); These targets were inhibited by dasatinib and quercetin, respectively (Zhu et al., 2015). Intriguingly, later, HSP90 inhibitors (Fuhrmann--Stroissnigg et al., 2017) and probably fisetin (Zhu et al., 2017) were identified to induce senolysis by inhibiting the PI3K pathway. Fisetin is a compound with a similar structure to quercetin and has been demonstrated to be a cell-type specific senolytic that alters multiple pathways (pro-survival PI3K is one of the pathways). Heat shock proteins like HSP90, have a critical role in maintaining proteostasis in cells (Somogyvári et al., 2022), therefore we postulate that it would be possible that HSP90 inhibitors exert their effect not only through inhibiting the pro-survival machinery of the cell but also through increasing intracellular proteotoxic stress.

A number of senolytics have been found to apply their effect through the regulation of upstream effectors of the Bcl-2 family proteins. In this review, we classify the regulators of Bcl-2 family proteins as the end point regulators of intrinsic apoptosis and will come back to it in the next section in detail. But now we focus on the indirect or the upstream regulators of these proteins. One of these upstream targets is tropomyosin receptor kinase B (TrkB) which has been shown to be pro-survival by activating Bcl-w and Extracellular Signal-Regulated Kinase 5 (ERK5); Small molecule inhibition of TrKB, induced apoptosis in senescent fibroblasts (Anerillas et al., 2022). In addition, gingerenone A, which has been shown to be senolytic in irradiation-induced senescent (IRIS) fibroblasts, reduces Bcl-xl expression in senescent cells probably by regulating the upstream pathways, which needs to be investigated thoroughly (Moaddel et al., 2022). Recently, cycloastragenol, a metabolite isolated from Astragalus membrananceus, has been shown to be senolytic which was associated with the reduction of Bcl-2 protein and the inhibition of its upstream, PI3K/AKT/mTOR pathway (however, the potency is not suitable for a drug since the effect was observed at the concentration of 100 μM, which is high) (Zhang et al., 2023).

One of the major regulators of apoptosis alongside cell cycle, at the upstream of the Bcl-2 family of anti-apoptotic proteins, is p53. P53 by inducing the expression of BH3 mimetics (including Bcl-2 Interacting Mediator of cell death (BIM), P53-Upregulated Modulator of Apoptosis (PUMA), and NOXA) has been implicated in the repression of the Bcl-2 family of anti-apoptotic proteins and consequent apoptosis stimulation. Nevertheless, p53 also participates in the activation of CDKN1A (p21) to abrogate cell cycle propagation in senescent cells (Aubrey et al., 2018). Interestingly, it seems that while p53 is overexpressed in the early stages of senescence induction, its expression is repressed in the late stages of senescence (Sturmlechner et al., 2022).

Different attempts have been made to increase the released p53 in senescent cells to provoke apoptosis. One of the approaches was to prohibit the proteasomal clearance of p53 following the ubiquitination by MDM2 either by increased degradation of MDM2 through Ubiquitin Specific Peptidase 7 (USP7) inhibition (using P5901 and P22077) (He et al., 2020a) or by direct inhibition of p53-MDM2 interaction (using UBX0101 small molecule) (Jeon et al., 2017). Of note, p53-MDM2 interaction inhibition by nutlin-3a induces a senescent-like phenotype in human fibroblasts which can be reversible depending on the oxygen concentration (Wiley et al., 2018); The response duality might be due to differences in the MDM2 site of action targeted by the inhibitors or genetic context variation in proliferative versus senescent cells. It is worth mentioning that the phase II clinical trial of UBX0101 in patients with moderate to severe painful osteoarthritis of the knee failed to meet the primary endpoint. Another successful pre-clinical approach is to interfere with the FOXO4-p53 protein interaction using the FOXO-DRI peptide (Baar et al., 2017). All these approaches led to an increased release of active p53 which has been proven to have a senolytic effect (Sturmlechner et al., 2022). Surprisingly, gingerenone A, a natural compound, apart from Bcl-xl suppression in senescent cells, which we pointed out before, has been shown to increase p53 level in both proliferative and senescent cells while it selectively kills senescent cells (Moaddel et al., 2022); It is congruent with the notion that the context-dependent variation in response to p53 elevation might be another underlying reason for their apoptotic versus non-apoptotic cell fate decision. Another upstream target of senescence-related terminal anti-apoptotic proteins is reported to be Signal Transducer and Activator of Transcription 3 (STAT3). Nintedanib by suppression of STAT3 elicited senolytic effect (Cho et al., 2022). The investigators claimed that Bcl-xl expression in senescent cells was not interfered with by nintedanib, however, other terminal regulators of apoptosis were not investigated in the study and remain to be in question.

2.1.2. Inhibition of end-point regulators of the intrinsic apoptosis pathway

Instead of targeting the upstream proteins of the end-point regulators of apoptosis, researchers from early on aimed to target the end-point regulators of apoptosis directly by senolytics. A couple of terminal anti-apoptotic proteins that are under the spotlight now for developing senolytics are the Bcl-2 family (Bcl-2, Bcl-xl, Bcl-w, and MCL-1) proteins. Targeting these proteins is seriously pursued and has resulted in

promising outcomes, although optimization of selectivity and efficacy of these drugs remain to be the hurdles throughout their development process. ABT-263 (navitoclax), and ABT-737 are the first and the most studied inhibitors of Bcl-2 and Bcl-xl proteins (Chang et al., 2016; Yosef et al., 2016). Navitoclax has been shown to be lethal in senescent human umbilical vein endothelial cells (HUVECs), IMR90, and mouse embryonic fibroblasts (MEFs) (Zhu et al., 2016). In addition, this drug has been shown to be able to clear senescent cells in vivo and attenuate age-related manifestations (Aguayo-Mazzucato et al., 2019; Chang et al., 2016; Walaszczyk et al., 2019). Nevertheless, the on-target effect of navitoclax and ABT-737 on Bcl-xl brings about thrombocytopenia in humans since this protein is the major anti-apoptotic protein that thrombocytes rely their survival on (Ashkenazi et al., 2017).

To overcome this dangerous adverse effect researchers have proposed various solutions. They have exploited specific Bcl-2 family inhibitors like ABT-199 with minimal inhibitory effect on Bcl-xl protein, yet, the outcome was not quite satisfactory as the senolytic effect was just observed in a small subset of senescence types (Yosef et al., 2016). Other researchers have suggested the indirect suppression of Bcl-xl activity by its enhanced proteolysis utilizing PROTAC technology, which delivers Bcl-xl (using the navitoclax binding moiety) to the E3 ligase cereblon (CRBN), to address the thrombocytopenia events. Notably, CRBN is poorly expressed in thrombocytes; Accordingly, they did enhance the senolytic effect while lowering the toxicity issue in non-senescent thrombocytes (He et al., 2020b). In a similar approach, an analog of curcumin, EF24 has been shown to promote proteasomal degradation of Bcl-2 and consequently senolysis in IRIS fibroblasts (Li et al., 2019). In senescent prostate tumor cells, MCL-1, another Bcl-2 family protein, has been found to have a greater role than other Bcl-2 family proteins in promoting resistance to apoptosis; And its inhibitor, S63845, was indicated to be a potent senolytic (Troiani et al., 2022). In a recent effort for computational discovery of senolytics, using deep learning networks, 3 compounds, BRD-K20733377, BRD-K56819078, and BRD-K44839765, were found to be senolytic in part by inhibiting Bcl-2 (Wong et al., 2023).

2.1.3. Inhibition of end-point regulators of the extrinsic apoptosis pathway

Bcl-2 family proteins and the upstream proteins are generally involved in the intrinsic induction of apoptosis, nevertheless, SASP has agents that can induce extrinsic apoptosis through TNF-α signaling either in a paracrine or autocrine manner, but the autocrine signaling must be abrogated as senescent cells can survive against this apoptotic stimulus. CFLAR (cFLIP) has been introduced as the major survival-maintaining target against this extrinsic pro-apoptotic trigger (Ivanisenko et al., 2022; Tsuchiya et al., 2015). It has been demonstrated that in the senescent cells, which have been primed for apoptosis by the upregulation of TNFRSF10B (DR5), the co-upregulation of cFLIP contributes to resistance against apoptosis. In consequence, the activation of DR5 with an agonist antibody, conatumumab, concurrent with the suppression of cFLIP by BRD2 inhibition using NEO2734 led to the senolysis of senescent cancer cells (Wang et al., 2022a). It is worth noting that NEO2734 is a dual BET and CBP/EP300 inhibitor with more complex targets, and in the study conatumumab and NEO2734 when used separately could not exert a significant senolytic impact.

2.1.4. Diminishing the pro-survival metabolites

Not only proteins but also metabolites are exploited by senescent cells to hinder apoptosis. The balance between ceramide and sphingosine/sphingosine-1-phosphate level is one of the emerging study areas for senescence versus apoptosis cell fate determination. N-Acylsphingosine Amidohydrolase 1 (ASAH1), which cleaves ceramide into sphingosine, is upregulated in senescent cells and its inhibition is senolytic in replicative senescent lung fibroblasts. Moreover, it enhances the senolytic efficacy of the cocktail dasatinib+quercetin when used in combination (Munk et al., 2021). It was also shown that Alkaline Ceramidase 2 (ACER2) upregulation, which catalyzes the hydrolysis of ceramides into sphingosine, increases the levels of both sphingosine and sphingosine-1-phosphate (S1P), while it decreases the level of ceramide in the cells. It has been demonstrated that just a moderate upregulation of ACER2 provokes cellular senescence by the elevation of S1P, whereas, its strong upregulation led to apoptosis (Xu et al., 2017). Therefore, it is probable that S1P is the pro-survival metabolite of senescence which levels up by a rate-limiting conversion from sphingosine. Consequently, when the production of sphingosine is inhibited or dramatically boosted, ceramide and sphingosine dysbalance can bring about apoptosis. Additionally, some new findings show distinct lipid profiles of senescent cells from our group (Narzt et al., 2021) and others (Wiley et al., 2021). Some of these lipids might have pro-survival roles and might be determined as senolytic targets.

2.2. Destabilization through enhanced stresses

Due to the suppression of many cellular functions related to repair and agile dynamic response to dysregulatory events in senescent cells, these cells cannot optimally counteract specific stress situations. Therefore, targeting senescent cells' weaknesses in stress management can selectively kill them while normal cells might easily tackle those specific stressors. Here we have collected some of these weaknesses that have been targeted by senolytics.

2.2.1. Defect in the establishment of proteostasis

One of the essential elements of cellular homeostasis is proteostasis, and its execution ensures the survival of a cell. A target found to flaw proteostasis is Crystallin Alpha B (CRYAB). CRYAB is a small heat shock protein that essentially forms soluble protein aggregates in eye lens. Blocking CRYAB aggregation by 25-hydroxycholestrol causes the accumulation of aggregates and proteotoxic stress which has been demonstrated not to be tolerable in senescent cells and brings about senolysis (Limbad et al., 2022). Moreover, senescent cells are incapable of establishing full proteostasis, per se (Sabath et al., 2020). Therefore, proteotoxic stresses in different forms either by disrupting the chaperoning system (including heat shock proteins which are selectively crucial in senescent cells) or by disturbing the functionality of the degrading system (proteasomal and autophagy system) which both cause lethal accumulation of proteins, might be efficient senolytic approaches in the future.

2.2.2. ROS accumulation and mitochondrial dysfunction

The exit of mitochondria from efficient oxidative phosphorylation and consequently the increased reactive oxygen species (ROS) generation have been known as one of the hallmarks of senescent cells (Martini and Passos, 2022; Miwa et al., 2022). Scavenging ROS (e.g. by peptide SS-31 or XJB-5–131) has been used in a number of studies to hinder accumulation of senescent cells in a preventive, not curative manner (Robinson et al., 2018; Sweetwyne et al., 2017), However, exacerbating ROS formation by targeting the respiratory chain in mitochondria of senescent cells has emerged as a strategy of senolysis which can curatively remove these cells. As an example, procyanidin C1 (PCC1), which is a polyphenol, in concentrations of more than 50 μM has been demonstrated to promote the production of ROS and mitochondrial dysfunction in senescent cells, leading to their senolysis in different cell types (WI-38, HUVEC, and Mesenchymal stem cells). The apoptosis induction was concurrent with the upregulation of NOXA and PUMA, denoting intrinsically induced apoptosis. PCC1 inhibited SASP in lower concentrations without any pro-apoptotic effect (Xu et al., 2021).

In the same line, derivatives of alkyl-monoquaternary ammonium-triphenyl phosphine (TPP) were studied for their senolytic effect. Alkyl-diquaternary ammonium-TPP with the longest chain length induced apoptosis in senescent cells selectively via the increase of ROS and mitochondrial membrane disruption (Jana et al., 2021). One of the most studied senolytics in this group is piperlongumine. Piperlongumine binds to oxidation resistance protein 1 (OXR1), a key player in oxidative

stress response, and induces OXR1 degradation through the ubiquitin proteasomal system (UPS) and eventually causes an elevation in ROS production and senolysis of senescent cells (WI-38 fibroblasts were studied) (Zhang et al., 2018). Oridonin, a natural terpenoid found in the traditional Chinese herbal medicine Isodon rubescens, has also been manifested to induce senolysis by targeting glutathione S-transferase and activating p-38 MAPK signaling (Zhang et al., 2022c); Glutathione S-transferase detoxifies endogenous oxidative reactive molecules by glutathione as a ROS scavenger. GPX4 is another protein that protects cells from oxidative damage and its defect is implicated in ferroptosis. Interestingly, it is indicated that despite GPX4 activity being defective in senescent cells, RSL3 which inactivates GPX4 can selectively induce a cell death associated with increased expression of transferrin, in senescent cells (Liao et al., 2022); this can be regarded as a kind of senolysis that mechanistically occurs through ferroptosis. Ultimately, the alkalization of lysosomes concurrent with an increased intracellular ROS by GL-V9 has been shown to exert a senolytic effect in senescent MDA-MB-231 cells (Yang et al., 2021).

2.2.3. Acidity and electrolyte imbalance

Increased intracellular acidity has long been known for its role in the induction of cellular apoptosis (Lagadic-Gossmann et al., 2004), however, just recently it has been implicated that senescent cells control intracellular pH to bring about resistance toward acid-induced apoptosis. One of the exploited mechanisms by senescent cells is the amplification of glutaminolysis through which the production of cytosolic ammonia can counteract any incident that might lead to a pro-apoptotic drop in pH (Johmura et al., 2021). Cardiac glycosides within two independent studies which interestingly were published at the same time manifested to be senolytic though increasing intracellular acidity (Guerrero et al., 2019; Triana-Martínez et al., 2019); The effect was mimicked by amiloride which inhibits Sodium/Hydrogen Exchanger 1 (NHE1), a critical membrane sodium/proton exchanger, and overexpression of NHE1 demonstrated to be protective in senescent cells. Cardiac glycosides primarily inhibit the Na^+/K^+ pump, however, the resulting electrolyte imbalance has not been shown to be senolytic. Instead, cardiac glycosides might depolarize the cell membrane which presumably contributes to cross-membrane proton dysregulation (Triana-Martínez et al., 2019).

Cardiac glycosides including proscillaridin a, ouabain and digoxin were senolytic in senescent A549 (senescence was induced by different chemotherapeutics), senescent BJ fibroblasts (induced by oncogenic Ras or H_2O_2) and senescent SK-MEL-103 cells (induced by Palbociclib). Moreover, digoxin was senolytic in replicative exhaustion-induced (RS) chondrocytes and IRIS IMR90 fibroblasts, nevertheless, it was not senolytic in mouse embryo fibroblasts, representing possible response variation depending on cellular context. Digitoxin, another cardiac glycoside provoked senolysis in a concentration close to the plasma concentration (20–30 nM) of cardiac patients who receive it, emphasizing the possible senolytic benefits of cardiac glycosides in human clinical settings (Guerrero et al., 2019). Interestingly, recently, two new cardiac glycosides, oleandrin, and periplocin, were discovered to be senolytic using machine learning. Oleandrin was found to be the most potent senolytic cardiac glycoside and exerts its effect at 10 nM concentration - which is a lower effective concentration in comparison to other senolytic cardiac glycosides - on oncogenic Ras-induced senescent (OIS), RS IMR90 cells, and etoposide-induced senescent A549 cells (Smer-Barreto et al., 2023). Calcium is another electrolyte that is extensively controlled between different cellular compartments. Endoplasmic reticulum calcium release to cytosol or mitochondria has been implicated in both apoptosis and senescence (Martin and Bernard, 2018; Orrenius et al., 2003), however, its balance with other constituents of the whole cellular system is what determines this senescence versus apoptosis fate. To our knowledge no senolytic has been suggested that exerts its effect through modulation of calcium signaling; Therefore, targeting calcium signaling might be a potential avenue for the identification of new senolytics.

2.2.4. Altered metabolic profile

Senescent cells indicate an altered metabolic profile including NAD^+, genetic materials (i.e., deoxynucleoside triphosphates (dNTPs)), and lipids (Wiley and Campisi, 2021). The increased accumulation of poly unsaturated fatty acid (PUFA) is an instance of changes in lipid metabolites (Wiley et al., 2021). Earlier we mentioned the possible protective effect of some of these components against apoptosis, nevertheless, triggering accumulation of even some neutral metabolites might selectively provoke toxicity and consequently cell death in senescent cells since these cells comprise a transformed enzymatic machinery for handling metabolites. Despite that, metabolic changes have not been exploited adequately yet for the development of senolytics either by inhibiting protective factors or by inducing stress.

2.2.5. Inefficiency in compensating disturbance in multiple signaling points at once

Inhibition of multiple pathways which are critical in the basal functioning of senescent cells has been shown to be a promising approach to boost lethal selectivity against senescent cells with minimal effect on non-senescent cells. R406, a SYK inhibitor, is a compound with a senolytic effect that carries out senolysis by affecting multiple targets including inhibition of Focal Adhesion Kinase (FAK) and p38 Mitogen-Activated Protein Kinase (MAPK) as well as increasing ROS generation (implementation of each of the FAK or p38 inhibition alone could not elicit senolytic effect). R406 did not affect the expression of anti-apoptotic Bcl-2 family proteins but could increase BIM pro-apoptotic protein (Cho et al., 2020). Moreover, the inhibition of the YAP-TEAD complex by verteporfin, which derepresses DDIT4, exerts a senolytic effect probably through reducing mTOR function but not solely (Anerillas et al., 2023) since inhibition of mTORC1 by rapamycin or blockage of mTORC1-mTORC2 by pan-mTOR inhibitors has not shown any senolytic effect. pan-mTOR inhibitors (not rapamycin) were just sensitizer when added to other senolytics (Xu et al., 2022). Hence, mTOR, especially mTORC2, stabilizes the general homeostasis of senescent cells; And if its loss accompanies any other homeostasis destabilizing event (but not alone), it might lead to catastrophic failure for senescent cells and induce system collapse or apoptosis of the cells.

2.3. Miscellaneous routes of senolysis

Due to the recent extensive efforts, several compounds have been discovered serendipitously or through large screenings to be senolytic, however, their detailed mechanisms of action have remained to be elusive and are not classified easily. One of these senolytics which has been attained through screening is Bromodomain and Extra-Terminal Domain (BET) family deactivators. BET family protein inhibitors (BETi) including JQ1 and OTX015, and BET degrader (BETd), ARV825, were demonstrated to be potent senolytics in RS and OIS IMR90 fibroblasts. The investigators strived to unravel these drugs' underlying mechanism of senolysis; They proposed that the concurrent DNA double-strand break (DSB) repair inhibition (by downregulation of xrcc4) and activation of autophagy (indicated by overexpression of p62 and MAP1LC3B) could contribute to senolysis in doxorubicin-induced senescent TIG-3 cells. They have shown that inhibition of autophagy by chloroquine or bafilomycin A1 could substantially reduce BETd-induced senolysis (Wakita et al., 2020). JQ1 and presumably other BETis have also been indicated in an independent study to kill bleomycin-induced senescent human dermal fibroblasts through ferroptosis (Go et al., 2021). This is truly interesting since ferroptosis might contribute to autophagic cell death (Gao et al., 2016). It is worth investigating whether BETis provoke ferroptosis in BRAF-induced senescent cells too. Consistently, prolonged autophagy by excessive degradation of p62 protein has been indicated to contribute to apoptosis of senescent cells which were induced by oxidative stress (H_2O_2);

Utilization of 3-MA, an autophagy inhibitor, could rescue the cells (Huang et al., 2022a). In contrast to the cytotoxic effect of autophagy in some senescent cells, a cytoprotective effect has also been reported; for instance, chloroquine and bafilomycin A each per se could trigger senolysis in BRAF-induced senescent BJ fibroblasts (but not in etoposide-induced senescent BJ fibroblasts) (L'Hôte et al., 2021).

PD0325901, a MEK inhibitor, has been demonstrated to be lethal in NaBut-induced senescent ERas-transformed rat embryonic fibroblasts; The lethality is reported to be due to the accumulation of ROS and damaged mitochondria. It has to be noted that in the study, the senescence inducer and the MEK inhibitor were used simultaneously without letting the senescence program to be fully implemented before the application of the MEK inhibitor; Making it possible that the authors have just assessed the effect of the MEK inhibitor in senescence accumulation (pre-senescence state) rather than its curative effect in the removal of previously senesced cells. Moreover, the authors have articulated that non-senescent ERas cells could overcome the lethality due to MEK/ERK suppression by cytoprotective autophagy which was not observed in the so-called senescent counterparts. Interestingly non-senescent ERas cells could overcome the temporary lethality of bafilomycin A1, but senescent ones could not get over this lethality and faced a permanent suppression of viability (Kochetkova et al., 2017). Accordingly, although the authors have pointed to mitochondrial damage and ROS accumulation as the main culprit of the senescent cell death, in reference to their own results, we think the process of the so-called senescent cell death here must be related to the general establishment of proteostasis by means of autophagy. Since cytoprotective autophagy is one of the legs of this proteostasis we speculate that in senescent cells in which at least one of the forms of autophagy (including chaperone-mediated, micro or macro autophagy) is impaired, cutting other legs of proteostasis could lead to the death of the cell. Consistently MEK has been implicated in the maintenance of proteostasis (Tang et al., 2015).

Collectively, different aspects of autophagy in senolysis are still under discussion and the occurrence of senolysis versus senolysis-protection triggered by autophagy inhibition probably depends on the whole survival machinery which is activated in that specific senescence type. Besides, the equivocal role of autophagy in senescent cell ablation or protection probably stems from the fact that autophagy has different classes, and each has its complexities. As a result, one cannot simply attribute outcomes related to the interference in one of the compartments to the whole process of autophagy. For example, from one side, degradation of KEAP1 (mediated by p62) and eIF3 by chaperone-mediated autophagy provoke redox homeostasis and proteostasis, respectively, which are crucial for combating stresses and presumably maintenance of cell survival in senescent cells (Lee et al., 2021). On the other side, progressive increase of macroautophagy concomitant with the increased degradation of p62, an autophagy compartment, could contribute to the apoptosis of senescent cells (Huang et al., 2022a). Therefore, it also seems that the outcome of autophagy manipulation depends on the intensity and the site of intervention. Probing into the detailed cascades of events during autophagy and probably its interconnection with UPS fosters the development of the next generation of senolytics.

We also would like to classify SASP regulation in the miscellaneous group as its role in senescent cell survival versus death, has not been well clarified and we could not find any solid data which demonstrates that SASP regulates cell death from a distinct process from those we classified for senolysis above. In fact, the first generation of senolytics were discovered in a quest to disable the anti-apoptotic machinery of senescent cells, which protects senescent cells from apoptotic stimuli including even their own pro-inflammatory SASP. SASP has different components out of which some promote cell death, especially pyroptosis, an inflammation-induced cell death, in neighbor normal cells through an unexplored mechanism that is overcome by HRas or ErbB2 oncogenic mutations. Whether this is also the autocrine mechanism of resistance of senescence cells against pyroptosis was not evaluated (Lisa et al., 2023). On the other hand, some other components of SASP have been shown to have an inhibitory effect on apoptosis induction presumably through NF-κB signaling activity (Salminen et al., 2012). Therefore, antagonizing the whole SASP from its master regulator site (including mTOR) might not result in a net effect of senolysis since pro-pyroptotic contents would also shrink. Nevertheless, targeting specific contents of SASP such as miRNAs in the small extracellular vesicles (sEVs) which have been indicated to exert an anti-apoptotic effect (Terlecki-Zaniewicz et al., 2018), might lead to discovering new senolysis pathways.

One of the target proteins that was found to be overexpressed on the surface of senescent cells which also causally is involved in induction of senescence is dipeptidyl peptidase 4 (DPP4) (Kim et al., 2017; Valencia et al., 2022). Although it might be used to specifically target ADCC or drugs towards senescent cells, directly inhibiting DPP4 has no clear senolytic effects, as DPP4 knock-down by siRNA in WI-38 fibroblasts resulted in increase of cell numbers and decrease of senescence markers (Kim et al., 2017). In human vascular smooth muscle cells, though, simultaneous treatment of the cells with a small molecule DPP4 inhibitor and a senescence inducer ($CoCl_2$ or Doxorubicin) induced apoptosis during senescence induction in vitro, and showed beneficial effects on atherosclerotic plaque stability in vivo (Herman et al., 2023). Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1B (DYRK1B) protein has recently been claimed to be a potential senolytic target in senescent endothelial cells. However, the root mechanism of its inhibition's action has not been cleared in senolysis (Pramotton et al., 2023). DYRK1B involves in many cellular processes related to cell survival including its identified interactions with MEK/ERK and mTOR, which makes it a precious target to be further studied in this realm (Boni et al., 2020). The other claimed senolytics are the fibrates class of drugs, like fenofibrate, which activates PPAR-α and autophagy, however, the mechanism of senolysis has been poorly understood (Nogueira-Recalde et al., 2019). Additionally, azithromycin and roxithromycin were senolytic in MRC-5 and BJ fibroblasts (Ozsvari et al., 2018). Interestingly, roxithromycin reduced ROS by inhibition of NOX4 which can be interpreted as a senomorphic effect, too (Zhang et al., 2021). CPG-74514A, a CDK1 inhibitor, in concentrations less than 10 μM, was selectively senolytic in Ras-induced and etoposide-induced senescent fibroblasts (Guerrero et al., 2019). Furthermore, zoledronate, a bisphosphonate, was senolytic in etoposide-induced senescent IMR90 fibroblasts, however, its mechanism of action was not explored (Samakkarnthai et al., 2023). Several agents have been reported to be senolytic just in senescent cancer cells; Panobinostat, a histone deacetylase inhibitor (HDACi), is senolytic in senescent non-small cell lung cancer (NSCLC) cells (Samaraweera et al., 2017). Interestingly, CUDC-907, another HDAC inhibitor that also inhibits PI3K, has been shown to induce apoptosis in a couple of senescent cancer cell lines, through modulation of multiple pathways, partially through HDAC6 inhibition and increasing p53 activation (Al-Mansour et al., 2023). Bortezomib, which is a proteasome inhibitor, selectively kills cisplatin-induced senescent H460 cells by enhancing ER stress (Ei et al., 2021). Paradoxically, bortezomib has been shown to be a senescence inducer in multiple studies but in different cells (Wang et al., 2022b). It emphasizes the need for further investigating the boundary for induction of senescence or killing senescent cells. An overview on all senolytics, their known/unknown mode of action as well as the model in which they have been discussed so far is provided in Table 1. Table 2 represents a number of senolytics with respect to their studied complications that have reached in vivo or clinical stages.

3. Heterogeneity of senolytic efficacy and cell specificity

From the early discovery of senolytics it became evident that due to differences in senescent cells and triggers, a senolytic effect is not universal and is cell-type specific or sometimes even senescence-trigger

Table 1
Classified senolytics, identified in different cell types by different senescence triggers.

Classes of senolytics	Molecule	Mechanism of action	Cell type	Senescence trigger	Reference
Inhibitors of end-point regulators of intrinsic apoptosis	Navitoclax	Bcl-2 inhibitor, Bcl-xl inhibitor	HUVECs[a], IMR90 MEFs[b]	Irradiation High oxygen passaging	(Zhu et al., 2016)
	ABT-737	Bcl-2 inhibitor, Bcl-xl inhibitor	IMR90	Etoposide, Oncogenic Ras	(Yosef et al., 2016)
	BRD-K20733377, BRD-K56819078, BRD-K44839765	Bcl-2 inhibitor	IMR90	Etoposide, Replicative exhaustion	(Wong et al., 2023)
	s63845	Mcl-1 inhibitor	Prostate tumor cells (PC3 shTIMP1 and LNCaP)	Docetaxel, Palbociclib	(Troiani et al., 2022)
	EF24	Promote proteasomal degradation of Bcl-2	WI-38	Irradiation	(Li et al., 2019)
	A1331852, A1155463	Bcl-xl inhibitor	HUVECs, IMR90	Irradiation	(Zhu et al., 2017)
Manipulators of upstream regulators of apoptosis	Fisetin	PI3K inhibition	HUVECs		
	Dasatinib	EFNB-1/3 inhibitor	Preadipocyte cells	Irradiation	(Zhu et al., 2015)
	Quercetin	PI3K inhibitor	HUVECs		
	Hyperoside	PI3K inhibitor	HUVECs	Replicative exhaustion	(Hwang et al., 2018)
	17-DMAG	HSP90 inhibitor, PI3K inhibitor	MEFs, Mouse MSCs[c] IMR90 WI-38	High oxygen passaging Etoposide Replicative exhaustion	(Fuhrmann-Stroissnigg et al., 2017)
	Gingerenone A, 6-shogalol	Bcl-xl inhibitor, Increase p53 level	WI-38	Irradiation	(Moaddel et al., 2022)
	UBX0101	Inhibitor of MDM2/p53 interaction, Increase p53 level	-	-	-
	FOXO4-DRI	Release active p53	IMR90	Doxorubicin, Irradiation	(Baar et al., 2017)
	P5901, P22077	USP7 inhibitor, Increase p53 level	WI-38, IMR90, Renal epithelial cells, HUVECs, Preadipocytes	Irradiation, Replicative exhaustion	(He et al., 2020a)
	Nintedanib	STAT3 inhibition	HDFs[d]	Replicative exhaustion	(Cho et al., 2022)
Disturbers of acidity and electrolyte balance	Proscillaridin, Ouabain, Digoxin	Intracellular acidity enhancer	A549	Bleomycin, Etoposide, Doxorubicin, Palbociclib, Gemcitabine	(Triana-Martínez et al., 2019)
			BJ Fibroblasts	Oncogenic Ras, H_2O_2	
			SK-mel 103	Palbociclib	
	Digoxin	Intracellular acidity enhancer	Chondrocytes IMR90	Replicative exhaustion Irradiation	
	Oleandrin	Intracellular acidity enhancer	IMR90	Replicative exhaustion, Oncogenic Ras	(Smer-Barreto et al., 2023)
			A549	Etoposide	
	Ouabain	Intracellular acidity enhancer	IMR90	Doxorubicin, Etoposide, Palbociclib, Oncogenic Ras	(Guerrero et al., 2019)
			Primary bronchial epithelial cells	Mid-passage	
Proteotoxic stressor	25-hydroxychloroquine	Inhibitor of aggregation of CRYAB	IMR90, Mouse dermal fibroblasts	Doxorubicin	(Limbad et al., 2022)
			hCMECs[e], hLSCs[f], hRPTECs[g], hACs[h]	Doxorubicin, Irradiation	
Disturbers of multiple signalings	R406	FAK and p38 inhibitor, Increase ROS generation	HDFs	Replicative exhaustion, Etoposide, H2O2	(Cho et al., 2020)
	Verteporfin	Derepress DDIT4 and reduce mTOR	WI-38	Etoposide	(Anerillas et al., 2023)
Oxidative stressors and disturbers of mitochondrial function	Procyanidin C1	Promote ROS production and mytochondria dysfunction	Human stromal cells	Replicative exhaustion, Oncogenic HRas, Bleomycin	(Xu et al., 2021)
			WI-38, HUVEC, MSCs	Bleomycin	
			PC27	Replicative exhaustion	
	Piperlongumine	OXR1 degradation, Elevation of ROS generation	WI-38	Replicative exhaustion, Irradiation	(Zhang et al., 2018)
	GL-V9	Alkalizes lysosome and Increase ROS	mda-mb-231 MEFs	Doxorubicin Replicative Exhaustion	(Yang et al., 2021)
	TPP[a] derivatives	Increase ROS and disrupt mitochondrial membrane	IMR90	Doxorubicin	(Jana et al., 2021)
	RSL3	Increase expression of transferrin and elevate ROS generation	PTEC[i]	Irradiation	(Liao et al., 2022)
	Oridonin	Activate ROS p-38 signaling	A549 L929 and HepG2	Cisplatin UVA irradiation	(Zhang et al., 2022c)
Miscellaneous	Bortezomib	Proteasome inhibitor, ER stress	H460	Cisplatin	(Ei et al., 2021)
	Azithromycin, Roxithromycin	-	MRC5, BJ fibroblasts	BrdU	(Ozsvari et al., 2018)
	Roxithromycin	-	WI-38	Irradiation, Bleomycin	(Zhang et al., 2021)

(*continued on next page*)

Table 1 (*continued*)

Classes of senolytics	Molecule	Mechanism of action	Cell type	Senescence trigger	Reference
	Panobinostat	Histone deacetylase inhibitor, Suppressor of Bcl-xL expression	NSCLC, HNSCC	Taxol, Cisplatin	(Samaraweera et al., 2017)
	CUDC-907	Histone deacetylase inhibitor; PI3K inhibitor, p53 activator	EJp53[j] H522, HCT116	TET removal Irradiation, Doxorubicin	(Al-Mansour et al., 2023)
	Ouabain, Chloroquine, Bafilomycin A	Autophagy inhibitor	BJ cells	Oncogenic BRAF	(L'Hôte et al., 2021)
	JQ1, OTX015, arv825	BET inhibitor/degrader, DSB repair inhibition and activation of autophagy	IMR90	Replicative exhaustion, Oncogenic Ras	(Wakita et al., 2020)
	JQ1	BET inhibitor, Ferroptosis inducer	HDFs	Bleomycin	(Go et al., 2021)
	Zoledronate	-	IMR90	Etoposide	(Samakkarnthai et al., 2023)
	Fenofibrate	PPAR-α activation	Chondrocytes	IL6	(Nogueira-Recalde et al., 2019)
	CGP-74514A	CDK1 inhibitor	IMR90	Oncogenic Ras, Etoposide	(Guerrero et al., 2019)

Abbreviations:
[*] Alkyl-monoquaternary ammonium-triphenyl phosphine (TPP).
[a] Human umbilical vein endothelial cells (HUVECs);
[b] Mouse embryonic fibroblasts (MEFs);
[c] Mesenchymal stem cells (MSCs);
[d] Human dermal fibroblasts (HDFs);
[e] Human cardiac microvascular endothelial cells (hCMECs);
[f] Human liver stellate cells (hLSCs);
[g] Human renal proximal tubule epithelial cells (hRPTECs);
[h] Human articular chondrocytes (hAC);
[i] Primary tubular epithelial cells (PTEC);
[j] p53 null EJ (EJp53);

specific. While dasatinib was able to selectively kill senescent preadipocytes, it was incapable of killing HUVECs. Of note, different behavior of HUVECs was also observed in the context of their apoptotic trait when senescent: they do undergo apoptosis over time as opposed to fibroblasts (Hampel et al., 2006; Wagner et al., 2001). On the other hand, quercetin was observed to act exactly the opposite of dasatinib concerning its activity on senescent preadipocytes and HUVECs (Zhu et al., 2015). Later it was determined that quercetin which was senolytic in HUVECs was not so in adult primary endothelial cells. Its derivative, hyperoside, behaved in the same way (Hwang et al., 2018). In this line, navitoclax which up to now has been shown to be senolytic in various senescent cell types was not selectively lethal in human primary preadipocytes (Zhu et al., 2016).

Fisetin, while senolytic in HUVECs (Zhu et al., 2017), did not impose senolysis in senescent primary human preadipocytes or senescent IMR90 fibroblasts, although in a different study, fisetin at concentrations roughly above 7.5 μM did kill senescent IMR90 cells. However, the therapeutic window was just selective for a very narrow concentration range, while higher doses were toxic to proliferative cells as well (Yousefzadeh et al., 2018; Zhu et al., 2017).

Digoxin, a commonly used cardiac glycoside, and a newly proposed senolytic, could not diminish the viability of senescent mouse embryo fibroblasts (Triana-Martínez et al., 2019). A recent study specifically delved into the difference in the apoptotic response of various senescent cells upon treatment with cardiac glycosides. Intriguingly, the authors articulated that the difference in apoptotic response is due to the existence of compensatory mechanisms for the regulation of potassium ion in the apoptosis-resistant senescent cells which were human mesenchymal stem cells (HMSCs). However, in apoptosis-prone cells including senescent IMR90 and A549 cells, they revealed that these cells were deprived of these compensatory means and were predisposed to apoptosis with overexpression of pro-apoptotic genes including BCL2 Associated Agonist of Cell Death (BAD), BCL2 Associated X Protein (BAX), BCL2 Related Ovarian Killer (BOK), BCL2 Antagonist/Killer 1 (BAK-1), and NOXA (Deryabin et al., 2021).

While in the mentioned study, authors just limited their research to non-oncogenic-induced senescence types, another study explored the senolytic mechanism of ouabain, one of the cardiac glycosides, in oncogenic BRAF-induced senescent fibroblasts. Surprisingly, they represented that the senolytic effect in BRAF-induced senescent cells is not provoked by potassium ion dysregulation but probably by autophagy inhibition. In congruence with this, they further showed that the inhibitor of autophagy, chloroquine, could induce apoptosis in BRAF-induced senescent cells (L'Hôte et al., 2021). However, the effect was not reproduced in etoposide-induced senescent cells, suggesting the ambiguous role of autophagy in senolytic response in senescence types of a same cell type but different senescence triggers.

Besides cell-type and trigger-type, recently, it has become apparent that the environmental state of the cell, which might vary in different tissues (Ortiz-Prado et al., 2019), can also control some aspects of senescence phenotype such as the role of oxygen supply in the intensity of SASP (van Vliet et al., 2021). Additionally, the availability of nutrients which is dependent on the diet can also affect senescence (Nehme et al., 2021). However, to what extent these variations can influence the anti-apoptotic pathways of the senescent cells is not well-known yet. This is significant as it might drastically transform the outcome of senolytic therapies clinically and has to be considered early in the studies since normal in-vitro studies are usually performed in a hyperbaric oxygen state. Moreover, it is almost impossible to develop senolytics exclusive for each senescence type, as the number of senescent cells depending on the cell-type, trigger-type, and even their physiological condition would be extremely high.

4. Combinatorial senolytics beyond dasatinib and quercetin

According to the previous section, the approach of one size fits all is most probably not achievable for senolytics due to the heterogeneity of senescence, and the respective different protective pathways (unless we find a pathway that is indeed specific to all senescent cells. Then it either must have an essential role in the survival of senescent cells or it might solely be used for targeting senescent cells by targeted delivery of cytotoxic components). As a result, scientists have been looking for ways

Table 2
Senolytics which have progressed beyond in vitro studies. Only latest stage references were included in the table for clarity reasons.

Senolytic	Latest study stage	Complication	Model	Publication
Dasatinib + Quercetin	Clinical	Idiopathic pulmonary fibrosis, Alzheimer's disease, Diabetic kindney disease	Human	(Gonzales et al., 2023;Hickson et al., 2020;Nambiar et al., 2023)
Fisetin	Clinical	Osteoarthritis	Human	Not published yet
UBX-0101	Clinical	Osteoarthritis	Human	(Lane et al., 2021)
Navitoclax	Preclinical in vivo	Age-related large elastic artery stiffening and endothelial dysfunction, Lung fibrosis, Pro-tumoral effects of obesity	Mice	(Clayton et al., 2023; Cooley et al., 2023; Fournier et al., 2023)
ABT-737	Preclinical in vivo	Age-related liver loss of regenerative capacity, Biliary injury after transplantation, Premalignant pancreatic intraepithelial neoplasia	Mice	(Dror et al., 2022;Ferreira-Gonzalez et al., 2022; Ritschka et al., 2020)
17-DMAG	Preclinical in vivo	Age-related symptoms	Mice	(Fuhrmann-Stroissnigg et al., 2017)
FOXO4-DRI	Preclinical in vivo	Age-related frailty, Age-related loss of renal function, Age-related testosterone secretion insufficiency	Mice	(Baar et al., 2017; Zhang et al., 2020)
25-hydroxychloroquine	Preclinical in vivo	Age-related aortic stiffness, Age-related muscle atrophy	Mice	(Limbad et al., 2022; Mahoney et al., 2023)
Verteporfin	Preclinical in vivo	Age-related immune cell infiltration, Fibrosis in aging mouse lungs	Mice	(Anerillas et al., 2023)
Procyanidin C1	Preclinical in vivo	Tumor, age-related physical dysfunction	Mice	(Xu et al., 2021)
Nintedanib	Preclinical in vivo	Lung fibrosis	Mice	(Cho et al., 2022)
Ouabain	Preclinical in vivo	Age-related local inflammation and immune infiltration, Tumor, Lung fibrosis	Mice	(Guerrero et al., 2019; Triana-Martínez et al., 2019)
RSL3	Preclinical in vivo	Aged kidney transplants	Mice	(Liao et al., 2022)
ARV825	Preclinical in vivo	Liver cancer, Lung fibrosis	Mice	(Sato et al., 2023; Wakita et al., 2020)
Fenofibrate	Pre-clinical, with retrospective human data analysis	Osteoarthritis	Human	(Nogueira-Recalde et al., 2019)

to extend the effect range of senolytics so that the effect incorporates at least a range of disease-related or tissue source-related senescent cells in vivo without generating toxicity on non-senescent cells.

Multiple approaches have been pursued up to now. The two most common were to combine senolytics or to add a sensitizer to a senolytic. These two approaches overlap sometimes because the sensitizer which is added to a senolytic to target a specific senescent cell is usually senolytic in different cells e.g., the combination of dasatinib and quercetin that both are senolytics, however, in distinct cells.

As mentioned in Section 2.2.2, mitochondrial function is impaired in senescent cells and their membrane potential declines. In a recent study, stressing mitochondria by further reducing the membrane potential selectively killed senescent fibroblasts in combination with navitoclax. Mitochondrial uncouplers (FCCP and BAM15) were used for this purpose. They neither induced senolysis alone, nor in combination with other senolytics tested including digoxin, dasatinib + quercetin or fisetin).(Edward et al., 2023).

ABT-199 is a selective Bcl-2 inhibitor which is more favorable in comparison to navitoclax in terms of not causing thrombocytopenia (Souers et al., 2013), however, since it does not inhibit Bcl-xl, it does not exert a significant senolytic effect, at least not in low doses (Rysanek et al., 2022; Yosef et al., 2016). Interestingly, in a recent study, Second Mitochondria-derived Activator of Caspases (SMAC) mimetics which inactivate IAPs, the inhibitor of apoptosis proteins, synergized with ABT-199 and selectively killed senescent IMR90, WI-38 cells, and senescent human retinal microvascular endothelial cells (HRMECs). Notably, the combination treatment of SMAC mimetics and navitoclax, which was systematically identified to be complementary, was a superior senolytic treatment rather than navitoclax alone. Parallelly, SMAC mimetics alone were not identified as potent senolytics (Colville et al., 2023).

In another example, the addition of a selective MCL-1 inhibitor, S63845, could enhance the senolytic effect of nonselective or selective non-MCL-1 Bcl-2 family inhibitors, potentiating administration of fewer doses to obtain less toxicity with a similar or even better efficacy (Rysanek et al., 2022). Intriguingly, in a different study, S63845 or navitoclax (a Bcl-2 family inhibitor) could not kill senescent melanocytes each separately, however, when they were combined, they could provoke senolysis. Furthermore, the authors declared that the protective effect of MCL-1 in apoptosis-resistant senescent melanocytes is due to upstream mTOR mediated MCL-1 translation; And mTOR inhibitor, PP242, could phenocopy the sensitizing effect of MCL-1 inhibition on senescent melanocytes (Kohli et al., 2022). mTOR is a central regulator of energy and nutrient sensing at the heart of many basic cell signalings. Direct inhibition of mTORC1 by rapamycin has not shown any senolytic or senolytic sensitizing effect perse, however, pan-mTOR inhibitors including PP242 and AZD8055 have been declared to sensitize senescent cells to senolysis through mTORC2 inactivation, and BIM overexpression in senescent mouse embryonic fibroblasts (NIH3T3) and senescent HUVECs (Xu et al., 2022). Together, to our knowledge, MCL-1 translation inhibition and mTORC2 inactivation are two possible senolytic sensitizing mechanisms proposed up to now, which are both implemented by pan-mTOR inhibitors. An overview of the combinatorial senolytic therapeutics, distinguished based on the individual senolytic or sensitizer effect in specific senescence models, has been represented in Table 3.

5. Prospective senolysis pathways

Most endeavors to discover senolytics have been dedicated to targeting the endpoint regulators of apoptosis (including Bcl-2 family proteins) and p53 signaling; Recently, mitochondrial function has also come into the focus. Despite that, unknowns regarding the role of autophagy, UPS, unfolded protein response (UPR), endoplasmic reticulum (ER) stress, and calcium signaling in the realm of senescence mechanism of apoptosis resistance prevail. Some studies demonstrate

Table 3
Combinatorial senolytic therapeutics, distinguished based on the individual senolytic or sensitizer effect in different senescence models (categorized by cell types and senescence triggers).

Senolytic combination	Senolytic	Sensitizer	Cell type	Senescence trigger	Reference
Dasatinib + Quercetin	Dasatinib Quercetin	Quercetin Dasatinib	Preadipocytes HUVECs[a]	Irradiation	(Zhu et al., 2015)
FCCP/BAM15 + Navitoclax	Navitoclax	FCCP/BAM15	HDFs[b], MRC5	Irradiation, Replicative exhaustion, Busulfan	(Edward et al., 2023)
Navitoclax/ABT-199 + Birinapant	Navitoclax/ABT-199	Birinapant	IMR90 WI-38 HRMEC[c]	Doxorubicin, Irradiation, H_2O_2, Bleomycin, Palbociclib, Etoposide Doxorubicin, Irradiation Doxorubicin	(Colville et al., 2023)
ABT-199/A1331852 + S63845	ABT-199/ A1331852	S63845	RPE-1	Irradiation	(Rysanek et al., 2022)
Navitoclax/ABT-737 + MIK665	Navitoclax/ABT-737	MIK665	RPE-1 BJ cells	Irradiation Docetaxel	
Navitoclax + S63845	-	S63845, Navitoclax	Melanocytes	Irradiation	(Kohli et al., 2022)
Navitoclax + PP242	-	Navitoclax, PP242			
Navitoclax + PP242/ AZD8055	Navitoclax	PP242/AZD8055	NIH3T3[d], HUVECs	H_2O_2, Adriamycin	(Xu et al., 2022)

Abbreviations:
[a] Human umbilical vein endothelial cells (HUVECs),
[b] Human dermal fibroblasts (HDFs),
[c] Human retinal microvascular endothelial cells (HRMECs),
[d] Mouse embryonic fibroblasts (NIH3T34).

apoptosis occurrence due to the manipulation of these processes. However, they are not well exploited for discovering selective cell-death inducers in senescent cells. Here we tried to gather some of the notable studies in this area which deserve to be paid attention to more.

It has been known for a couple of years that lysosomes with respect to their enzymatic capacity are defective in senescence cells, however, it has recently been shown that the increased autophagosome-lysosome biogenesis by Transcription Factor EB (TFEB)/ Transcription Factor E3 (TFE3) nuclear localization is the compensatory mechanism of these cells for survival and it has been demonstrated that the knockdown of TFEB/TFE3 causes senescent cell death. Therefore, alongside the manipulation of the execution of autophagy, interventions in early step biogenesis of autophagosomes and lysosomes might also lead to the development of senolytics (Curnock et al., 2023).

In addition, calcium regulation between cytosol, ER, mitochondria, and probably lysosomes in an interplay with UPR specifically due to calcium-dependent stresses, determines apoptosis versus survival-maintenance fate of senescent cells. Calcium signaling in terms of an increased calcium release from ER and its accumulation in mitochondria has substantial similarity in both apoptosis and senescence (Martin and Bernard, 2018; Orrenius et al., 2003; Wiel et al., 2014; Ziegler et al., 2021). However, it is articulated that overexpression of proteins like CALB1 which buffers the detrimental effect of calcium release, contributes to apoptosis resistance in senescent cells (Raynard et al., 2022). Therefore, targeting calcium buffering processes in senescent cells can potentially be an alternative approach for developing senolytics. Furthermore, a study demonstrated that dramatic IP3R1-mediated calcium release led to cell apoptosis which was rescued by ERN1 expression (Son et al., 2014). Intriguingly, ERN1 overexpression is implicated in the promotion of OIS cells (Blazanin et al., 2017). We speculate that ERN1-mediated UPR is a pro-survival mechanism utilized by senescent cells against the pro-apoptotic effect of ER calcium depletion and can be disrupted to achieve new types of senolytics. Apart from the removal of senescent cells by targeting their internal regulatory machinery, some recent techniques have been utilized for alternative targeting of these cells. Some of these techniques are seeking for the enhancement of immune system ablation of senescent cells in vivo either by vaccination against surface proteins of senescent cells (Suda et al., 2021), immune activation by glycolipid antigens (Arora et al., 2021), by CAR-T cell therapy (Amor et al., 2020; Yang et al., 2023), or even by assistive antibodies which break the resistance of senescent cells against immune clearance (Wang et al., 2022c). Other techniques are established based on senescence-specific biomarker targeted delivery of cytotoxic compounds (Ekpenyong-Akiba et al., 2019; Guerrero et al., 2020; Muñoz-Espín et al., 2018). We avoid further delving into these alternative methods' mechanisms of action since their selectivity or implementation is provided by a second-hand system e.g., a delivery system or immune system. Intriguingly, recent studies articulate the possible senolytic effect of specific types of exercise. If this comes to be true through more investigations, this would also open a new horizon for mimicking a physiological senolysis route (Chen et al., 2021; Jean et al., 2023) (an overview of the classified mechanisms of senolytics is available in Fig. 1).

6. Concluding remarks

In summary, the ultimate goal of senolysis in a clinical setup is not just killing senescent cells but it is to enhance the net performance of the whole organism. Therefore, to reach an advantageous senolytic therapy we need to consider the molecular mechanisms of senolysis and the whole organismal context whether it is ageing, cancer, fibrosis, etc., within which we want to exert this effect. It must be noted that in the context of aging, many cellular functionalities even in non-senescent cells are declined similar to senescent ones. For instance, mitochondrial function (Jang et al., 2018) and cellular proteostasis establishing systems (Hipp et al., 2019) become more inefficient and if we utilize stressors including the mitochondrial disturbers, the proteotoxic stresses, and the others that we classified earlier as the means of senolysis, we might be able to selectively kill senescent cells, however, we also might impair the overall performance of non-senescent aged cells too. In such a scenario, the off-target side effects might be less when using protection repressors, specifically the ones with minimal effect on other hallmarks of ageing (López-Otín et al., 2023).

Another unsolved issue is the timing of senolytic treatment. One might argue that senolytics should be consumed with time intervals so that the effects on non-senescent cells will be limited in time, but further investigations will be necessary to clarify this. Additionally, the on-target elimination of senescent cells has to be carefully evaluated for adverse effects, as the loss of too many cells might be worse than persistent senescent cells. In addition, senescent cells have a variety of

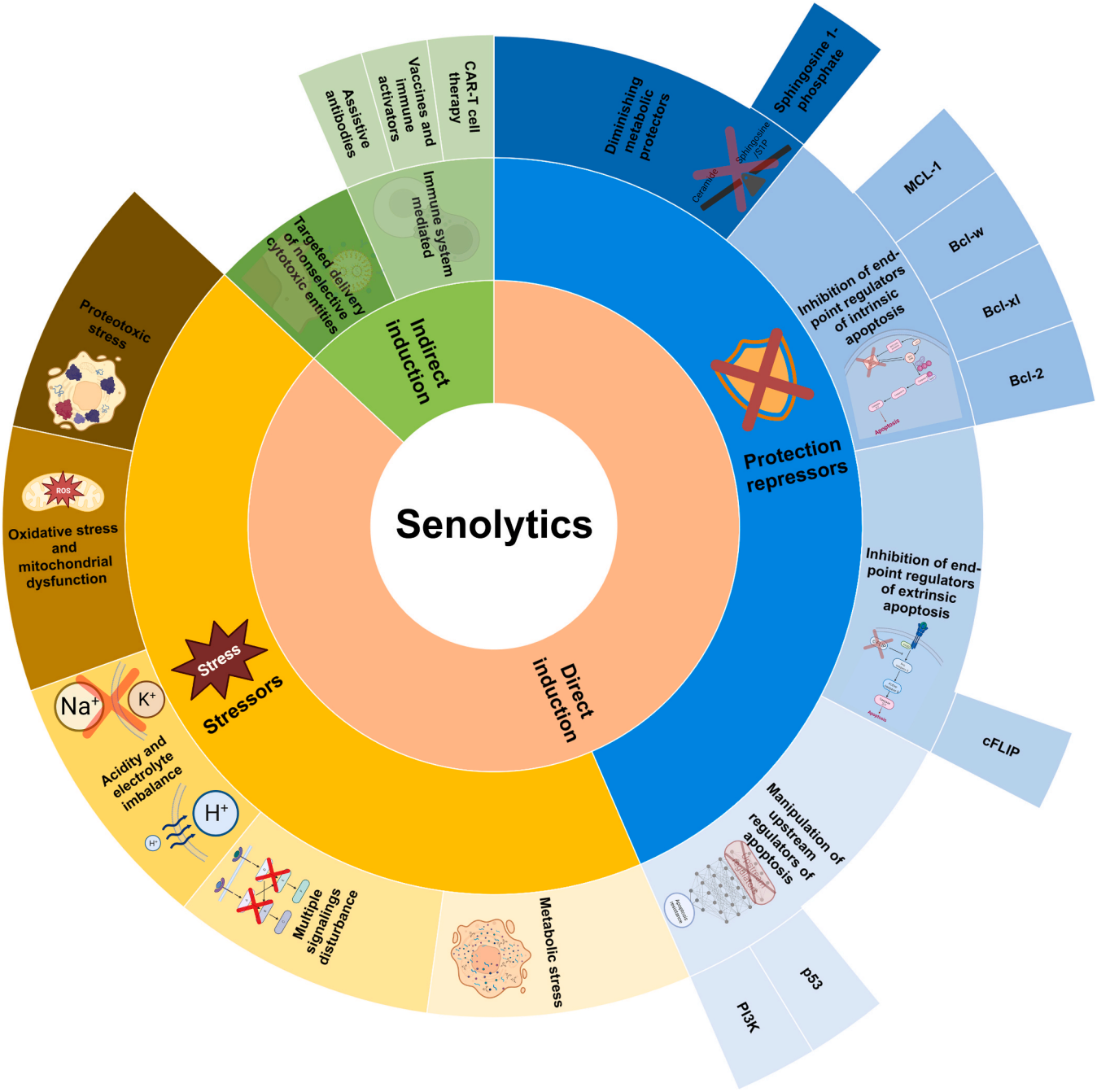

Fig. 1. Illustrative overview of the classified mechanisms of senolytics (created with BioRender.com).

beneficial, physiological roles including in wound healing (Demaria et al., 2014), oncogenesis surveillance (Campisi, 2005; Kang et al., 2011), immunogenicity (Marin et al., 2023), embryogenesis (Muñoz-Espín et al., 2013), and cellular reprogramming (von Joest et al., 2022). Interfering with these physiological functions potentially brings about health risks that can restrain senolytics usage in certain populations. It must be evaluated whether the benefits outweigh the risks to general health (Raffaele and Vinciguerra, 2022).

Still, many of the drugs now ranging among the senolytics had been popular for their health benefits before being identified as senolytics, especially the natural ones, like polyphenols, cardiac glycosides, and piperlongumine. It remains to be clarified whether these effects were indeed always due to senolysis. Finally, there is a need to conduct unconventional research including the utilization of machine learning techniques for the development of the next generations of senolytics. At the same time, elucidation of underlying pro-survival machineries of senescent cells and discovery of lead agents through large screenings, along with the facilitative role of computational methodologies will further help to rationally develop senolytics. Computational approaches and large screenings can also be helpful for assessment of the combination of senolytics or their combination with sensitizers to understand if they can broadly target multiple cell types in a tissue or multiple tissues at once preferably with lower risks. Just then, we might achieve a groundbreaking therapeutic which brings drastic breakthroughs in the therapy of many diseases associated with the detrimental effect of senescence – senopathies.

CRediT authorship contribution statement

Amirhossein Nayeri Rad: Conceptualization, Investigation, Writing

– original draft, Writing – review & editing. **Johannes Grillari:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

Johannes Grillari is co-founder and shareholder of Rockfish Bio AG. Amirhossein Nayeri Rad has no competing interests to declare.

Data Availability

Data will be made available on request.

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